# Advantages of Superficially Porous Particles (SPP) for Faster HPLC and UHPLC Separations

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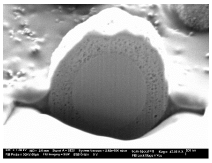
Presented at HPLC 2019 Poster: 448

## Introduction

- Parallel efforts by James Jorgenson who developed sub-2µm, fully porous particles (FPP) [1] and Jack Kirkland who went in a new direction with superficially porous particles (SPP) [2] now allow much faster HPLC experiments to be carried out.
- Low, flat van Deemter plots for the latest FPP and SPP column technology show large efficiency improvements and opportunities to develop fast, new QC methods and also revalidate methods that already employ HPLC without the high pressure consequences.
- 2 µm SPP column technology delivers resolution and speed advantages for optimized UHPLC and LC-MS methods.

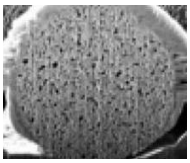
## The Unique Superficially Porous Particle (SPP)

Superficially Porous Particle (SPP)



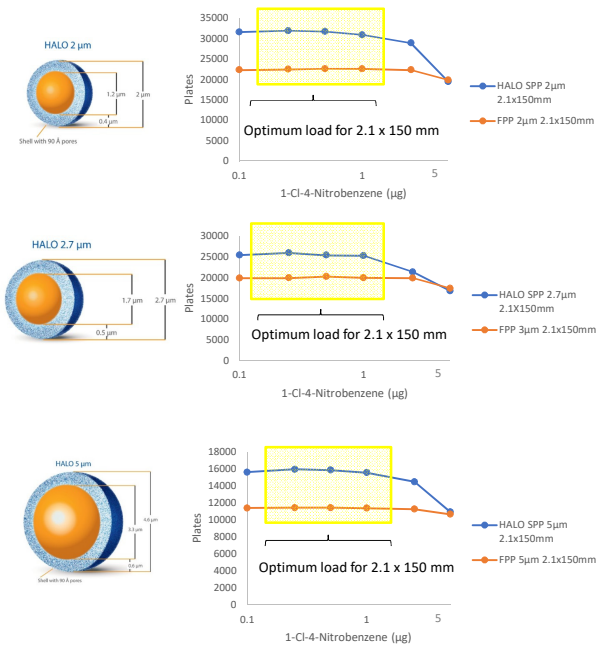
- Highest purity solid silica core surrounded by porous shell
- Tightly controlled, highly uniform particle manufacturing process

Fully Porous Particle (FPP)



- Porous silica structure throughout
- Single process technique subject to more variability

## SPP vs. FPP Sample Loading Comparison

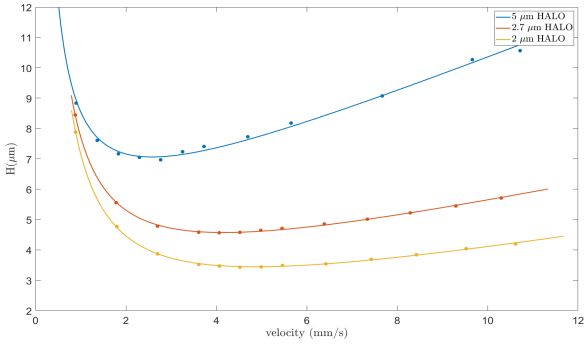



## Performance Plots for 5, 2.7, and 2 µm SPP Columns

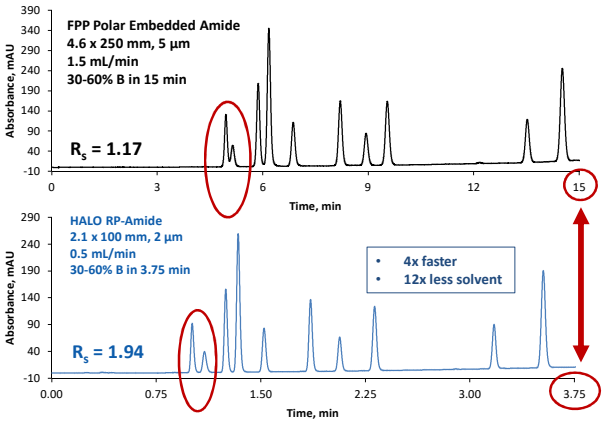
**van Deemter Equation**

H = height equivalent to theoretical plate
A = eddy diffusion term
B = longitudinal diffusion term
C = resistance to mass transfer term
µ = mobile phase linear velocity ($L/t_0$)

$$H = A + \frac{B}{u} + Cu$$




## SPP Advantages: Resolution Improvement, Time Savings, and Solvent Savings




$$R_s = \frac{\sqrt{N}}{4} \cdot \frac{k_2}{(k+1)} \cdot \frac{(\alpha-1)}{\alpha}$$




PEAK IDENTITIES:
1. homovanillic acid
2. caffeic acid
3. syringic acid
4. vanillic acid
5. chlorogenic acid
6. sinapic acid
7. ferulic acid
8. *p*-coumaric acid
9. *trans*-cinnamic acid
10. resveratrol

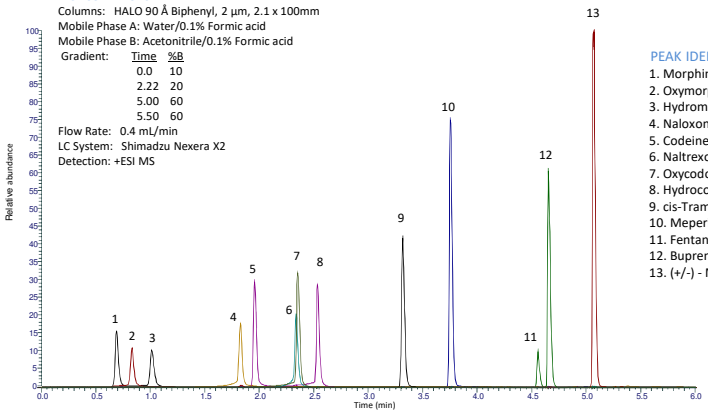
The purpose of improving efficiency is to maintain or increase resolution. Small particle sizes, such as 2 µm with shorter lengths deliver efficiency quickly, making them an ideal choice for optimized high throughput analysis. Notice that the separation above is x4 faster on the SPP particle compared to the FPP, saving not only time but solvent.

## LC-MS Separation of Pain Management Opiates

TEST CONDITIONS:
Columns: HALO 90 Å Biphenyl, 2 µm, 2.1 x 100mm
Mobile Phase A: Water/0.1% Formic acid
Mobile Phase B: Acetonitrile/0.1% Formic acid
Gradient:

| Time | %B |
|---|---|
| 0.0 | 10 |
| 2.22 | 20 |
| 5.00 | 60 |
| 5.50 | 60 |

Flow Rate: 0.4 mL/min
LC System: Shimadzu Nexera X2
Detection: +ESI MS




PEAK IDENTITIES:
1. Morphine (m/z 286)
2. Oxymorphone (m/z 302)
3. Hydromorphone (m/z 286)
4. Naloxone (m/z 328)
5. Codeine (m/z 300)
6. Naltrexone (m/z 342)
7. Oxycodone (m/z 316)
8. Hydrocodone (m/z 300)
9. cis-Tramadol (m/z 264)
10. Meperidine (m/z 248)
11. Fentanyl (m/z 337)
12. Buprenorphine (m/z 468)
13. (+/-) - Methadone (m/z 310)

The 2 µm HALO Biphenyl is an ideal choice for high throughput analysis of drug panels, in which isobaric species separation is needed. Note the resolution between codeine and hydrocodone, (peaks 5 and 8, respectively) and morphine and hydromorphone (peaks 1 and 3, respectively).

## Optimization Consideration to Achieve the Best 2 µm Performance

- Optimized systems are necessary to see the most benefit from high efficiency columns, specifically SPP's of smaller diameter such as the 2 um.
- The following considerations are the most common and easiest to implement:
  - System Volume:
    - Minimize excess tubing and install appropriately sized id tubing for the UHPLC and/ or LCMS system
    - If using an LC detector, such as UV, choose a small flow cell volume (<1 µl ideally)
    - Consider inherent LC system volumes resulting from the pumping configuration (binary vs. quaternary vs. isocratic), injector design (fixed loop vs. in-flow)
  - Data Collection Considerations:
    - LC Detector Sampling Rate- 20 Hz minimum, higher preferred
    - MS Scan Speed- as fast as possible considering targeted or untargeted analysis
- Overlooked items that compromise column efficiency
  - Improper pore size
  - Improper connections
  - Improper column dimension for LC instrument
  - Improper injection volume and solvent

## Conclusions

- 2 µm particles deliver speed and increased efficiencies with the use of modern and optimized UHPLC and LC-MS systems.
- Due to SPP performance at lower operating back pressures compared to FPP, longer column lengths can be used when high resolution is required.
- Short SPP columns can be easily adopted for fast, high throughput LC-MS assays of complex mixtures where the MS resolving power can be exploited.
- Development of new SPP columns and technologies continues with new particle geometries, pore sizes and bonded phase chemistries to further the scope of their application.

1. J. W. Jorgenson, et. al., LCGC N. A., Vol. 21, Number 7, July 2003, 600-610.
2. J. J. Kirkland, et. al., American Laboratory, 39 (February 2007), 18-21.